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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	John Kisiday et al.	Art Unit:	1614
Serial No.:	09/778,200	Examiner:	Not yet assigned
Filed:	February 6, 2001	Customer No.:	21559
Title:	PEPTIDE SCAFFOLD ENCAPSULATION OF TISSUE CELLS AND USES THEREOF		

Assistant Commissioner for Patents
Washington, D.C. 20231

Version with Markings to Show Changes Made

A marked-up version of Table 1 on page 19, line 1, through page 20, line 9, of the specification is presented below.

Table 1. Representative Self-Assembling Peptides

Name	Sequence (n-->c)	Modulus	Structure	SEQ ID NO
RADA16-I	n-RADARADARADARADA-c	I	β	1
RGDA16-I	n-RADARGDARADARGDA-c	I	r.c.	2
RADA8-I	n-RADARADA-c	I	r.c.	3
RAD16-II	n-RARADADARARADADA-c	II	β	4
RAD8-II	n-RARADADA-c	II	r.c.	5
EAKA16-I	n-AEAKAEAKAEAKAEAK-c	I	β	6
EAKA8-I	n-AEAKAEAK-c	I	r.c.	7
RAEA16-I	n-RAEARAEARAEARAEA-c	I	β	8
RAEA8-I	n-RAEARAEA-c	I	r.c.	9
KADA16-I	n-KADAKADAKADAKADA-c	I	β	10
KADA8-I	n-KADAKADA-c	I	r.c.	11
EAH16-II	n-AEAEAHAHAEAEAHAH-c	II	β	12
EAH8-II	n-AEAEAHAH-c	II	r.c.	13
EFK16-II	n-FEFEFKFKFEFEFKFK-c	II	β	14
EFK8-II	n-FEFKFEFK-c	I	β	15
ELK16-II	n-LELELKLKLELELKLK-c	II	β	16
ELK8-II	n-LELELKLK-c	II	r.c.	17
EAK16-II	n-AEAEAKAKAEAEAKAK-c	II	β	18
EAK12	n-AEAEAEAEAKAK-c	IV/II	α/β	19
EAK8-II	n-AEAEAKAK-c	II	r.c.	20
KAE16-IV	n-KAKAKAKAEAEAEAEA-c	IV	β	21
EAK16-IV	n-AEAEAEAEAKAKAKAK-c	IV	β	22
RAD16-IV	n-RARARARADADADADA-c	IV	β	23
DAR16-IV	n-ADADADADARARARAR-c	IV	α/β	24
DAR16-IV*	n-DADADADARARARARA-c	IV	α/β	25
DAR32-IV	n-(ADADADADARARARAR)-c	IV	α/β	26
EHK16	n-HEHEHKHKHEHEHKHK-c	N/A	r.c.	27
EHK8-I	n-HEHEHKHK-c	N/A	r.c.	28
VE20*	n-VEVEVEVEVEVEVEVEVEVE-c	N/A	β	29
RF20*	n-RFRFRFRFRFRFRFRFRFRF-c	N/A	β	30

"β" denotes beta-sheet; " α" denote alpha-helix; "r.c." denotes random coil; "N/A" denotes not applicable. *Both VE20 and RF20 form a beta-sheet when they are incubated in a solution containing NaCl; however, they do not self-assemble to form macroscopic scaffolds.

A marked-up version of Table 2 on page 29, lines 17 to 25, of the specification is presented below.

Table 2. Representative Peptides for Cross-Linking Study

Name	Sequence (N-->C)	SEQ ID. NO.
RGDY16	RGDYRYDYRYDYRGDY	31
RGDF16	RGDFRFDFRFDFRGDF	32
RGDW16	RGDWRWDWRWDWRGDW	33
RADY16	RADYRYEYRYEYRADY	34
RADF16	RADFRFDFRFDFRADF	35
RADW16	RADWRWDWRWDWRADW	36

A marked-up version of Table 3 on page 22, lines 14 to 21, of the specification is presented below.

Table 3. Representative Peptides for Enzymatic Cleavage Study

Name	Sequence (N-->C)	SEQ ID NO
REEE	RGDYRYDYTFREEE-GLGSRYDYRGDY	37
KEEE	RGDYRYDYTFKEEE-GLGSRYDYRGDY	38
SELE	RGDYRYDYTASELE-GRGTRYDYRGDY	39
TAQE	RGDYRYDYAPTAQE-AGEGPRYDYRGDY	40
ISQE	RGDYRYDYPTISQE-LGQRPRYDYRGDY	41
VSQE	RGDYRYDYPTVSQE-LGQRPRYDYRGDY	42

A marked-up version of the paragraph on page 23, line 22, through page 24, line 14, of the specification is presented below.

"A peptide with the amino acid sequence n-KLDLKLDLKLDL-c (SEQ ID NO. 43) (KLD12) was synthesized using a peptide synthesizer (Applied Biosystems) and lyophilized to a powder. A 0.5% peptide casting solution was obtained by dissolving KLD12 in a solution of 295 mM sucrose and 1 mM HEPES. Freshly isolated chondrocytes from bovine calf femoropatellar groove cartilage were re-suspended in the casting solution at a concentration of 15 x 10^6 cells/ml. The suspension was injected into a

casting frame consisting of a 40 x 40 x 1.5 mm window supported on both faces by filter paper and a porous mesh. The casting frame was placed in a 1 X phosphate-buffered saline (PBS, which contains 150 mM NaCl and 10 mM sodium phosphate at pH 7.4) bath for 15 minutes to induce the self-assembly of the peptides into a scaffold. Preferably, the cells are incubated in the sucrose solution for less than 5 minutes, or more preferably for less than 1 minute, before PBS is added. If desired, formation of a peptide scaffold may be confirmed using phase-contrast microscopy. As a control, cells were also suspended into warm agarose (2% solution, w/w), injected into the casting frame, and placed into a cold 1 X PBS bath for 5 minutes. Both the peptide and control agarose gels were maintained in DMEM media (Gifco) plus 10 % FBS, which was changed every other day."